



## **Fossil carbon is a major contributor to cellular biomass in geochemically distinct shallow fractured carbonate-rock aquifers**

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Despite the global significance of subsurface biosphere, the functioning of subsurface bacterial biomass and their relationships with surface are still poorly understood. Because sedimentary organic carbon can be the largest potential C source for micro-organisms isolated from surface reduced C, a better knowledge of the processes that affect its subsurface dynamics is essential to understand the functioning of subsurface microbial communities and their potential interactions with the global C cycle. In pristine shallow aquifers of limestone/marlstone alternations with different subsurface-surface interactions and functional bacterial diversities (Hainich Critical Zone Exploratory, central Germany), we determined the major microbial C sources by comparing the natural abundance of  $^{13}\text{C}$  ( $\delta^{13}\text{C}$ ) and  $^{14}\text{C}$  ( $\Delta^{14}\text{C}$ ) in phospholipid derived fatty acid (PLFAs) with potential microbial C sources including DIC, particulate organic carbon (POC) and methane ( $\text{CH}_4$ ). We showed that despite the presence of younger and thus potentially more labile C sources, a large fraction of the bacterial communities was using fossil organic substrates to varying degrees;  $\approx 33\%$  in oxic zones,  $\approx 47\%$  in iron-reduction zones and  $\approx 70\%$  in sulfate-reduction/annamox zones. In oxic groundwater, compound specific radiocarbon analyse of 11MeC16:0, a biomarker for nitrite oxidizing bacteria, supports a major autotrophic C pathway as its  $\Delta^{14}\text{C}$  value ( $-283\text{‰} \pm 32\text{‰}$ ) was similar to that of dissolved inorganic carbon (DIC) from the same well ( $-366\text{‰} \pm 18\text{‰}$ ). In anoxic groundwaters, the occurrence of sulphate-reduction on fossil organic substrates (sedimentary organic matter or hydrocarbons) was supported by the strong  $^{14}\text{C}$ -depletion of 10MeC16:0 ( $-942\text{‰} \pm 22\text{‰}$ ). Results indicated that the average  $^{14}\text{C}$ -content of microbial biomass in carbonate-rock aquifers results from complex interactions between abundance and bioavailability of naturally occurring organic matter, hydrogeology and specific metabolism of microorganisms.